STUDY OF DETECTION LIMITS AND QUANTITATION ACCURACY USING 300 MHZ NMR

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ABSTRACT

The analytical precision and accuracy of 300 MHz nuclear magnetic resonance (NMR) spectrometers was determined. The instruments that were used include a Bruker AC-300, a Bruker Avance 300, and a Varian 300. The minimum detection limits for proton (^{1}H) and phosphorus (^{31}P) were determined and compared for different instruments. The Minimum Detection Limits (MDL) for proton measurements were in the range of 3-10 μg/g. The MDL for phosphorus measurements were in the range of 50-70 μg/g. The quantitation accuracy for purity determinations was measured to be in the range of 1% relative to an internal standard. Specific MDL values should be determined for each particular matrix, internal standard, and instrument conditions.

INTRODUCTION

The analytical accuracy and precision of 300 MHz Nuclear Magnetic Resonance (NMR) spectrometers were studied under typical sample analysis conditions. Instruments used were Bruker Avance, Bruker AC-300, and Varian 300 MHz. Low concentration trace detection and high concentration purity determinations of chemical weapons simulants were done. Minimum detection limits (MDL) based on the 99% confidence limits were determined using the EPA criteria[1] for ^{1}H and ^{31}P NMR in standard solutions and aqueous samples.

EXPERIMENTAL METHODS

The sample or standard solutions were transferred to a 5 mm NMR tube to run on the 300 MHz NMR instruments. Unless otherwise stated, the same sample was analyzed on two days with 8 repetitions each day to determine the reproducibility of the NMR signals. Data processing was done with Acorn NUTS software, Bruker XWIN-NMR, or Varian software. Standard deviations of the data were used to determine 99% confidence limits, which were used for the MDL determinations.

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SAMPLE PREPARATION

An internal standard (IS) of hexamethylphosphoramide (HMPA, 99% purity) from Sigma Aldrich was added as a quantitation reference internal standard (IS). IS and samples were weighed. Dilute standards were diluted by weight to weight solvent. Typically, the IS was added at 10 wt.% of the sample or standard. This was used to obtain an accurate IS and solution weight. By comparison, this IS weight is much higher than the dilute analytes at 50-200 μg/g (ppm), giving an indication of instrumental dynamic range.

RESULTS

MDL FOR ^{1}H IN A STANDARD (AC-300)

The MDL was determined for proton NMR on the Bruker AC-300 with a standard solution. A standard of 45 ppm thiodiglycol (TDG) in D_2O was prepared. Water solvent suppression was used. The number of scans that were signal averaged was 128. As shown in Table 1, the measured MDL was 10 ppm. Recovery was 97%, indicating good quantitation accuracy.

Table 1. MDL Results for ^{1}H standard (AC-300)

Prepared Concentration (ppm)	45.81
Average Found Concentration (ppm) relative to internal standard	44.45
Standard deviation	3.95
Calculated MDL (ppm)	10.27
Recovery %	97%

MDL FOR ^{1}H IN A STANDARD (VARIAN 300)

For comparison, the MDL was also determined for proton NMR on the Varian 300. A standard solution of 45 ppm thiodiglycol (TDG) in D_2O was prepared. Water solvent suppression was used. As shown in Table 2, the MDL was 3 ppm, lower than the AC-300. Recovery was 96%.

Table 2. MDL Results for ^{1}H in Varian 300

Prepared concentration (ppm)	44.32
Average Found Concentration (ppm)	42.68
Standard deviation (ppm)	1.03
Calculated MDL (ppm)	3.09
Recovery %	96%

MDL FOR ^{1}H: EFFECT OF WEIGHING ERROR (VARIAN 300)

The MDL was determined for multiple standard preps using proton NMR on the Varian 300 to determine the error associated with weighing. Seven standard solutions of 45 ppm TDG in D_2O were prepared and analyzed for seven reps each. The average standard deviation for the seven NMR reps was 0.99 ppm in the measurement of the found concentration. The standard deviation for the seven different standards was 0.28 ppm, which indicates the error from weighing is less than the error from the NMR data repetitions.

Table 3. MDL for ^{1}H: Results for effect of weighing error (Varian 300)

Std. Dev. Average	0.990
Std. Dev. Deviation	0.276
Recovery Average	94.46%
Recovery Std. Dev.	1.58%
MDL Average	2.961
MDL Std. Dev.	0.824

MDL FOR ^{1}H IN AN AQUEOUS SAMPLE

An aqueous sample was studied without water suppression on the AC-300. The solution was mostly H_2O. The sample was spiked to 200 ppm with TDG. The same methodology was used. As shown in Table 4, the MDL was higher, at 116 ppm, due to interference from the large peak tailing of the water peak. The tailing made accurate integration of the peak more difficult, so recovery was only 76%. This is a worst case, but these results indicate the MDL can vary significantly depending on sample and instrument conditions. Of course, the MDL can also depend on the instrument parameters such as number of scans, excite pulse power, and excite pulse length.

Table 4. MDL Data for ^{1}H in an Aqueous Sample

Prepared concentration	202.30
Average Found Concentration (ppm)	153.97
Standard deviation	44.62
MDL (ppm)	116.11
Recovery %	76%

MDL FOR ^{31}P IN AN AQUEOUS SAMPLE

The MDL for ^{31}P NMR was determined for both AC-300 and Avance instruments, shown in Table 5 and 6. A standard solution of 100 ppm of diisopropyl methylphosphonate (DIMP) in H_2O was prepared. The major source of error was from the phasing of the spectra. The IS peak was much larger than the DIMP peak, which made it difficult for both peaks to have the optimal phasing. Small changes in the large peak made much larger effects on the integration of the nearby small peak. As a result, it is likely that the MDL for ^{31}P detection can be made significantly lower, similar to those for ^{1}H, by using less internal standard or by choosing an internal standard that is further away from the analyte peak.

Table 5. MDL Results for ^{31}P in a Standard for the AC-300

Prepared concentration (ppm)	99.48
Average Found Concentration (ppm)	98.06
Standard deviation	26.20
MDL (ppm)	68.18
Recovery %	98%

Table 6.MDL Results for ^{31}P in a Standard for the Avance

Prepared concentration (ppm)	99.48
Average Found Concentration (ppm)	100.44
Standard deviation	20.54
MDL (ppm)	53.45
Recovery %	101%

MDL is slightly higher for the AC-300 that for the Avance, which is a newer instrument, but there is not a large difference for the analysis of the same sample.

QUANTITATIVE PURITY DETERMINATIONS

NMR is used for purity determinations of agent standards, as well as trace analysis. A detailed comparison of quantitation accuracy and precision was made between the two Bruker 300 MHz instruments for each of the nucleii ^{1}H, ^{31}P, and ^{13}C. A sample was prepared by weighing about approximately equal amounts of HMPA (99% purity) and triethylphosphate (TEP, 99+% purity) and transferring it to an insert in a 5 mm NMR tube.

Results that are shown in Figure 1 were corrected for measured weights and molecular weights so the expected relative purity is 1.00. The measured accuracy for both instruments is within 1% of the expected purity.

Although the AC-300 typically had somewhat larger standard deviations, there was generally good agreement between the AC-300 and the Avance instruments. The one exception was case B, in which the excitation frequency was not centered between the two large phosphorus peaks. The AC-300 gave a much lower result due to the roll-off of the excite waveform.

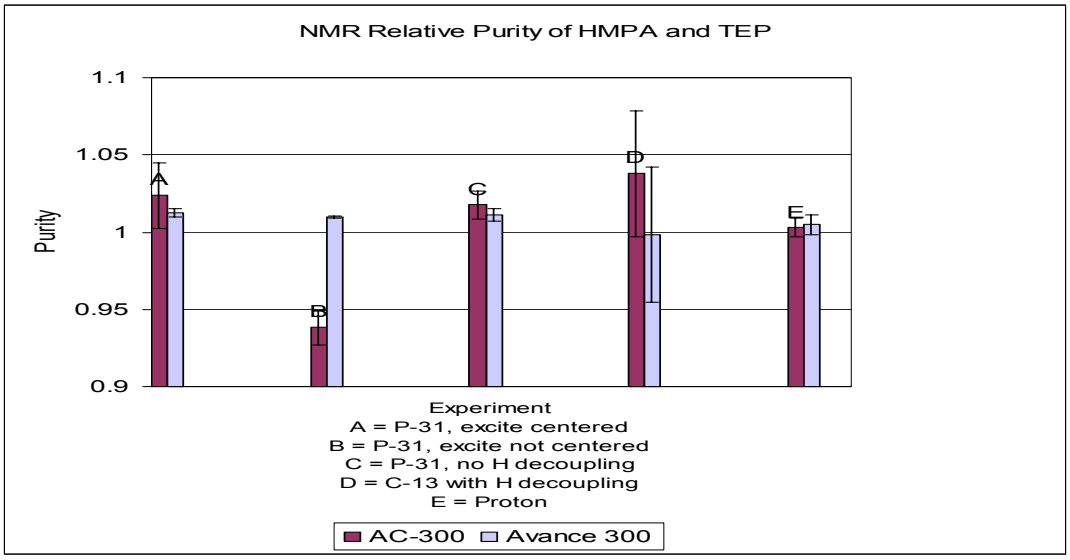

Figure 1. Quantitative Purity Determination Results for the comparison of the AC-300 to the Avance spectrometers. Error bars indicate one standard deviation from seven reps except for case B, which had three reps.

CONCLUSIONS

Although the older AC-300 instrument has slightly poorer performance, all three 300 MHz instruments give signal precision and accuracy that is reasonably similar. MDL results for proton measurements were in the range of 3-10 ppm in concentration, although the MDL depends on instrument conditions. MDL for phosphorus determinations were in the range of 50-70 ppm. Purity determinations gave accuracy in the range of 1% or less. Additional information is given in technical reports.[2-5]

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REFERENCES

1. Code of Federal Regulations, 40 CFR 136 Appendix B.

2. R. J. O'Connor, M. D. Brickhouse, D. J. McGarvey, H. D. Durst, W. R. Creasy, J. L. Ruth, "NMR Method for the Quantitative Purity Analysis of Neat Feed Stock Sample," ECBC Technical Report #ECBC-TR-253, August 2002.

3. R. J. O'Connor, M. D. Brickhouse, D. J. McGarvey, W. R. Creasy, J. L. Ruth, H. D. Durst, "NMR Method for the Quantitative Analysis of Neat Materials," ECBC Technical Report Draft, October 2002.

4. D. J. McGarvey, R. J. O'Connor, M. D. Brickhouse, W. R. Creasy, J. S. Rice, J. Pence, J. L. Montgomery, H. D. Durst, "NMR Method for the Quantitative Analysis of Trace-level Analytes in Liquid Samples," ECBC Technical Report Draft, October 2002.

5. D. J. McGarvey, R. J. O'Connor, M. D. Brickhouse, W. R. Creasy, J. S. Rice, H. D. Durst, "NMR Method for the Quantitative Analysis of VX Hydrolysates," ECBC Technical Report Draft, October 2002.